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Original Article

Use of Bentofeed and Persian Melon Peel Biochar in the Decolorization of Water Contaminated with Methylene Blue and their Effects on *In vitro* Ruminal Fermentation

Kazemi, M[1] *, Mokhtarpour, A[2]

1. Department of Animal Science, Faculty of Agriculture and Animal Science, University of Torbat-e Jam, Torbat-e Jam, Iran
2. Research Center of Special Domestic Animals, University of Zabol, Zabol, Iran

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Corresponding Author: phd1388@gmail.com

Abstract

This study conducted two experiments to evaluate the effects of two cheap adsorbents, including bentofeed (a commercial name of bentonite) and Persian melon peel biochar (PMPB) on the decolorization of water contaminated by methylene blue (MB) and ruminal fermentation pattern. The decolorization efficiency of bentofeed and PMPB at three levels of 0, 4, and 8 mg per 10 ml of 0, 3, 6, and 9 mg/L MB solutions mg/L after 3 and 24 h of incubation was evaluated by its absorbance at 660 nm. At all dye concentrations, PMPB, and bentofeed showed high potential in removing MB from water with an efficiency of 60%-99.5%. In both incubation times, the addition of 8 mg bentofeed had the highest effect on the removal efficiency when the dye concentration was 6 or 9 mg/L. However, the removal efficiency was declined with increasing MB concentration ($P<0.05$). Experiment two evaluated the effects of various levels (same as experiment one) of MB, bentofeed, and PMPB on *in vitro* gas production (GP) and volatile fatty acids (VFA) in two individual 4×3 factorial experiments. The potential GP (b), rate constant of gas production (c), metabolizable energy, organic matter digestibility, and total VFA were significantly decreased with increasing MB in the medium ($P<0.05$), while all parameters were increased when bentofeed or PMPB was added to the medium containing MB ($P<0.05$). The amounts of acetic, propionic, and butyric acids were not affected by PMPB; however, they changed when bentofeed was added to the medium ($P<0.05$). The NH_3-N concentration was decreased significantly following the increase of MB; moreover, it was increased when PMPB and bentofeed were added to the medium. MB, as a water contaminant agent, had negative effects on ruminal fermentation parameters. Both adsorbents (i.e., PMPB and specially bentofeed) were efficiently able to remove MB from the water. The negative effects of MB on fermentation parameters were also alleviated as a result of using bentofeed or PMPB. It seems that bentofeed has the higher adsorption property of MB, compared to that of the PMPB.

Keywords: Bentofeed, Biochar, Fermentation, Gas production

Utilisation de la Bentofeed et du Biochar de la Pelure du Melon Persan dans la Décoloration de l'Eau Contaminée au Bleu de Méthylène et Leurs Effets sur la Fermentation Ruminale *In Vitro*

Résumé: Cette étude a mené deux expériences pour évaluer les effets de deux adsorbants bon marché, dont la Bentofeed (un nom commercial de la bentonite) et le Biochar de la Pelure du Melon Persan (BPMP) sur la décoloration de l'eau contaminée par le bleu de méthylène (BM) et le modèle de fermentation ruminale. L'efficacité de décoloration de la Bentofeed et du BPMP à trois niveaux de 0, 4 et 8 mg par 10 ml de 0, 3, 6 et 9 mg/L de solutions BM mg/L après 3 et 24 h d'incubation a été évaluée par son absorbance à 660 nm. À toutes les concentrations de colorant, le PMPB, et la Bentofeed ont montré un potentiel élevé d'élimination du BM de l'eau avec une efficacité de 60% à 99.5%. Dans les deux temps d'incubation, l'addition de 8 mg de la Bentofeed a eu

l'effet le plus élevé sur l'efficacité d'élimination lorsque la concentration de colorant était de 6 ou 9 mg/L. Cependant, l'efficacité d'élimination a été diminué avec l'augmentation de la concentration de BM (P<0.05). La deuxième expérience a évalué les effets de divers niveaux (identiques à la première expérience) de BM, de la BPMP et de la Bentofeed sur la production de gaz (PG) in vitro et les acides gras volatils (AGV) dans deux expériences factorielles individuelles 4 × 3. La PG potentielle (b), la constante de vitesse de production de gaz (c), l'énergie métabolisable, la digestibilité de la matière organique et le total des AGV ont été considérablement réduits avec l'augmentation du BM dans le milieu (P<0.05), tandis que tous les paramètres ont été augmentés lorsque la Bentofeed ou la BPMP ont été ajouté au milieu contenant BM (P<0.05). Les quantités d'acides acétique, propanoïque et butyrique n'ont pas été affectées par la BPMP; cependant, elles ont changé lorsque la Bentofeed a été ajoutée au milieu (P<0.05). La concentration de NH_3-N a diminué de manière significative suite à l'augmentation du BM; de plus, elle a été augmentée lorsque de la BPMP et de la Bentofeed ont été ajoutés au milieu. Le BM, en tant qu'agent de contamination de l'eau, avait des effets négatifs sur les paramètres de fermentation ruminale. Les deux adsorbants (c'est-à-dire la BPMP et spécialement la Bentofeed) ont pu efficacement éliminer le BM de l'eau. Les effets négatifs du BM sur les paramètres de fermentation ont également été atténués grâce à l'utilisation de la Bentofeed ou de la BPMP. Il semble que la Bentofeed ait la propriété d'adsorption du BM plus élevée que celle de la BPMP.
Mots-clés: Bentofeed, Biochar, Fermentation, Production de gaz

1. Introduction

Various chemical dyes are widely used in industries, such as textile, rubber, paper, plastic, and cosmetic that produce a large volume of wastewaters. These wastewaters are rich in colour and contain harmful chemicals some of which are toxic, mutagenic, and carcinogenic (Sun et al., 2013; Kelm et al., 2019). Every year, a large number of dyes are produced in Iran's textile industries which may contaminate the water resources and can be consumed by the livestock.

Among various physical, chemical, and biological decolorization techniques, treatment with activated carbon is an effective and attractive physical process due to its high efficiency (Kelm et al., 2019). However, the use of activated carbon is restricted due to the high costs of its production. Therefore, there is an increasing interest in finding renewable and effective alternative low-cost adsorbents for the decolorization of wastewaters.

Biochar is a pyrolytic product of organic waste, typically trees, forages, straws, and agricultural waste, under the partial or complete absence of oxygen (Sun et al., 2013) which can absorb water pollutants, such as dye (Sumalinog et al., 2018) from the soil. More recently, biochar has been applied in ruminant feed (Schmidt et al., 2017) because of its ability in reducing enteric methane emissions (Leng et al., 2012) improving animal health and feed efficiency (Schmidt et al., 2017).

Persian melon (*Cucumis melo* CV. Khatooni) is one of the most important agricultural products in Iran with an annual production of about 1.47 million tons (IAAS, 2015). Bentonite containing aluminosilicate compounds is also another suitable source of adsorbent material for the adsorption of chemical dyes from aqueous solutions (Liu et al., 2015). It should be noted that about 2.5% of the world's bentonite is produced in Iran. Due to the lack of sufficient information about the impacts of MB on ruminal fermentation and the probable consumption of contaminated waters by livestock, it is hypothesized that bentonite and biochar produced from melon peel may reduce the possible adverse effect of MB on ruminal fermentation *in vitro*. This study aimed to evaluate the adsorption potential for removing MB from an aqueous solution using bentonite and melon peel biochar; moreover, it was attempted to investigate their effects on *in vitro* ruminal fermentation.

2. Material and Methods

2.1. Preparation of Dye and Adsorbents

The Persian melon fruits (*Cucumis melo* CV. Khatooni) were collected randomly from the farms of

Torbat-e Jam, Iran, located at 35°23N′latitude, 60°64E′ longitude. The peels were separated and washed to remove dust, chopped into smaller pieces, and then transferred to an oven at 60°C for 48 h. After drying, the slices of melon peel were ground, passed through mesh No. 400, and placed in the chamber. Subsequently, the pyrolysis was carried out in a homemade electric heated pyrolysis reactor at about 550°C for 3 h. Before the pyrolysis, the reactor was purged with nitrogen gas at 10 psi for 15 min to ensure the absence of oxygen during the reaction (Sumalinog et al., 2018).

Bentonite, a combination of montmorillonites with the commercial name of bentofeed, was purchased from the Vivan group (Mashhad, Iran), ground, and passed through mesh No. 200. Furthermore, Bentofeed was washed with distilled water several times, filtered, and then dried in an oven at 60°C for 48 h.

2.2. Dye Decolorization

In total, four levels of MB (0, 3, 6, and 9 mg/L) with three levels of PMPB and bentofeed as sorbents (each at 0, 4, and 8 mg) were used in two separate 4×3 factorial experiments to measure the removal ability of dye from water. Afterward, 10 ml of MB (prepared from stock solution) with PMPB or bentofeed were added to the special tubes and incubated in a water bath at 39°C for 3 or 24 h. For each treatment, five replications were considered. At the end of the incubation time, the tubes were centrifuged at 3500×g for 15 min, and the absorbance was read using a spectrophotometer (Photonix-Ar-2017) at 660 nm. The removal percentage of MB (R) was calculated according to the following equation:

$$R\ \% = [C0 - Ce/C0] \times 100$$

where C0 and Ce are the initial and final concentrations of the MB (after adsorption), respectively.

2.3. *In vitro* Gas Production

In vitro gas production was measured according to Menke and Steingass (1988). The rumen fluid was collected from three male Moghani sheep fed on a ration containing alfalfa and concentrate (60:40). This fluid was then strained through four layers of cheesecloth and flushed with CO_2. About 200 mg of 1 mm milled alfalfa (dry matter basis) were transferred into 100 ml calibrated glass syringes. In the next stage, three levels of bentofeed or PMPB (0, 4, and 8 mg, equivalent to 0, 2, and 4% of substrate DM) were added to the syringes. Afterward, four concentrations of MB (0, 3, 6, and 9 mg/L) prepared in 30 ml of rumen-buffer mixture (1:2, v/v) were added to the pre-warmed (39°C) syringes containing the sample. All syringes were put in a water bath maintained at 39°C. The incubation was carried out in four replicates within each run, and each run was replicated. Gas production volume was recorded at 3, 6, 9, 12, 24, 48, 72, and 96 h. After 24 h of incubation, the contents of two syringes from each treatment were filtered through four layers of cheesecloth. After that, 5 ml of 0.2N HCl was equally combined with 5 ml of filtered rumen fluid for the determination of NH_3-N. Another subsample of 5 ml of filtered rumen fluid was mixed with 1 ml of 25% meta-phosphoric acid and then preserved at -20°C for VFA analysis.

The kinetic of gas production parameters were estimated according to the model of Ørskov and McDonald (1977) as:

$$P = b\,(1 - e^{-ct})$$

where P is the volume of gas production at time t, b signifies the gas production from an insoluble but fermentable fraction (ml/200 mg DM), c denotes the rate constant of gas production for b (%/h), and t presents the incubation time (h). Organic matter digestibility (OMD) and metabolizable energy (ME) were estimated based on Menke and Steingass (1988) equations for roughage feeds as follows:

$$ME\ (MJ/kg\ DM) = 2.20 + 0.1357\ GP + 0.057\ XP + 0.0029\ XL^2;$$

$$OMD\ (\%) = 15.38 + 0.8453\ GP + 0.595\ XP + 0.675\ XA$$

where GP, XP, XL, and XA are the gas productions at 24-h incubation for 200 mg DM of the sample, crude protein (g/kg DM), crude fat (g/kg DM), and crude ash (g/kg DM), respectively.

2.4. Laboratory Analysis

After thawing, rumen fluid was centrifuged (Eppendorf AG, Hamburg, Germany) at 3000×g for 20 min at 4°C, and the VFAs concentrations were then determined by gas chromatography (Philips PU-4410) equipped with a flame ionization detector and a semi-capillary TR-FFAP (30 m×0.53 mm×1 m) column (Supelco, USA). The temperature of 140°C was considered for the column, and 250°C was considered both in the injector and detector. The flux of helium as carrier gas was 13 mL/min.

2.5. Statistical Analysis

A 4×3 factorial experiment (the first factor was four levels of MB, and the second factor was three levels of adsorbent) was applied for data analysis using PROC GLM of SAS software (Version 9.1, SAS Institute). The statistical model was:

$$Y_{ijk}=\mu+A_i+B_j+AB_{ij}+\varepsilon_{ijk}$$

where Y_{ijk} is the dependent variable, μ signifies the overall mean, A_i denotes the effect of MB levels, B_j presents the effect of adsorbent levels (bentofeed or PMPB), AB_{ij} indicates the interaction between MB levels and adsorbent levels, and ε_{ijk} is the residual error. It is worth mentioning that the differences between treatment means were determined by Duncan's multiple range test at $P<0.05$.

3. Results

The removal efficiency of MB in water by bentofeed and PMPB at 3- and 24-h of incubation is shown in Figure 1 and Figure 2, respectively. Bentofeed and PMPB were able to remove MB at three concentrations (3, 6, and 9 mg/L) with high efficiency ranged from 60% to 99.5%. In the concentration of 3 mg/L, more than 91% of MB was removed by the inclusion of bentofeed and PMPB. The removal efficiency of both adsorbents was declined by increasing the concentration of MB at both incubation times. The maximum efficiency of MB removal was observed when 8 mg bentofeed was added to the medium containing 6 or 9 mg/L MB. The lowest removal efficiency was observed in 4 mg PMPB when it was dosed at 6 and 9 mg/L ($P<0.05$).

The effect of MB with or without bentofeed on gas production and estimated parameters are presented in Table 1. The estimated parameters including b, c, OMD, and ME were significantly decreased when MB increased in the medium up to 6 mg/L ($P<0.05$). The b, ME, and OMD were increased by the addition of bentofeed in all concentrations of MB ($P<0.05$).

Table 2 shows the effect of MB with or without PMPB on gas production and estimated parameters. The value of b was decreased by the inclusion of 8 mg PMPB, compared to the control group ($P<0.05$). Neither 4 mg nor 8 mg PMPB had any effect on c, ME, and OMD, compared to the control group ($P>0.05$). The values of b, ME, and OMD were increased when 8 mg PMPB was added to the medium containing MB ($P<0.05$).

The inclusion of 8 mg bentofeed decreased NH_3-N and TVFA, compared to the control group ($P<0.05$) (Table 3). By increasing the levels of MB up to 6 mg/L, TVFA and molar proportion of acetic acid were decreased; however, the propionic acid was increased ($P<0.05$). Nonetheless, butyric acid was not affected by treatments ($P>0.05$). The lowest concentration of NH_3-N was observed in 9 mg/L MB ($P<0.05$), and the inclusion of bentofeed significantly increased TVFA and NH_3-N concentration in MB containing treatments ($P<0.05$).

As shown in Table 4, the molar proportion of acetic, propionic, and butyric acids were not affected by PMPB ($P>0.05$); however, TVFA and NH_3-N contents were decreased, compared to the control group. The addition of PMPB to the medium containing MB significantly increased TVFA and NH_3-N concentration ($P<0.05$).

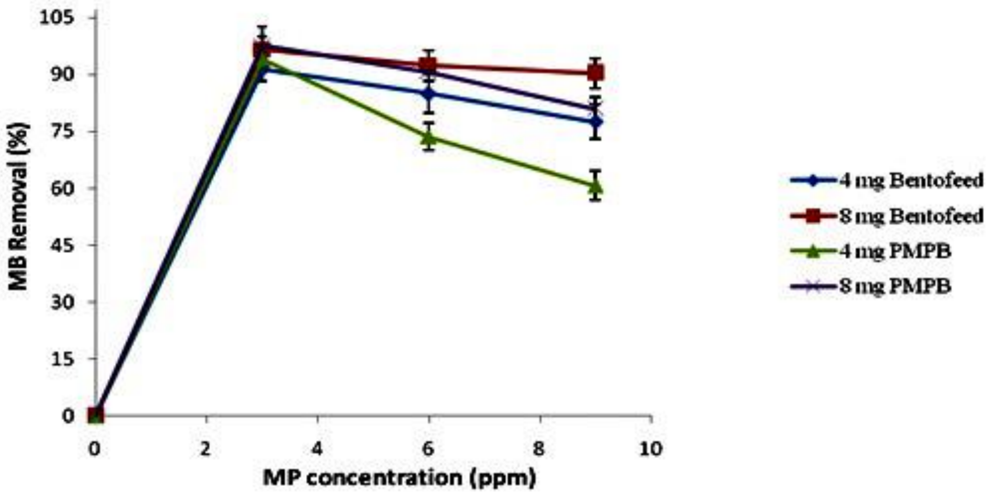

Figure 1. Removal efficiency of methylene blue (MB) in water by bentofeed and Persian melon peel biochar (PMPB) at 3-h incubation time

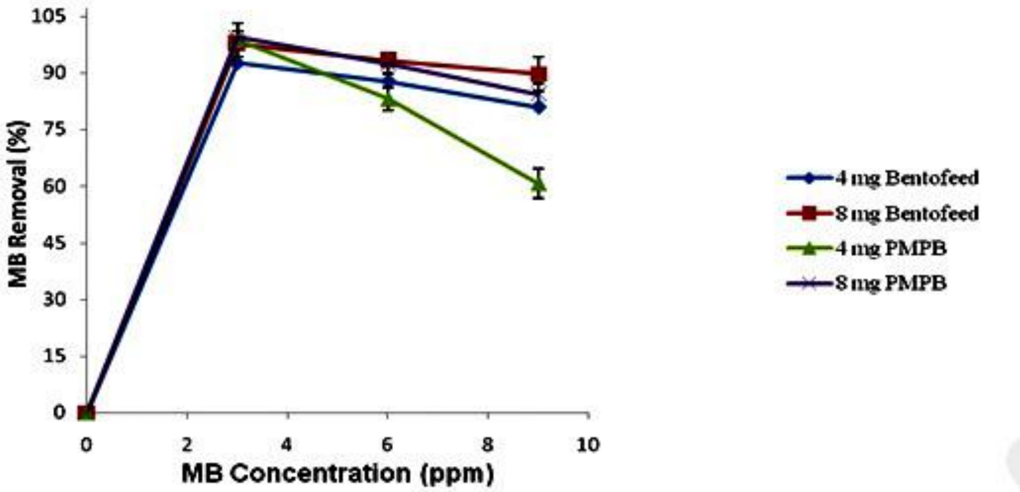

Figure 2. Removal efficiency of methylene blue (MB) in water by bentofeed and Persian melon peel biochar (PMPB) at 24-h incubation time

Table 1. Effect of methylene blue with or without bentofeed on the gas production and estimated parameters

Treatment	b	c	ME	OMD
Control	72.02^{a}	0.055^{b}	9.29^{a}	70.43^{a}
Control+4 mg bentofeed	68.09^{a}	0.064^{a}	9.26^{a}	70.23^{a}
Control+8 mg bentofeed	68.56^{a}	0.054^{b}	8.92^{a}	68.50^{a}
3ppm MB	23.90^{c}	0.026^{cd}	3.98^{cde}	43.06^{cde}
3ppm MB+4 mg bentofeed	28.17^{bc}	0.027^{cd}	4.24^{bc}	44.36^{bc}
3ppm MB+8 mg bentofeed	30.80^{b}	0.032^{c}	4.61^{b}	46.26^{b}
6ppm MB	18.67^{d}	0.027^{cd}	3.67^{de}	41.42^{de}
6ppm MB+4 mg bentofeed	24.96^{c}	0.022^{d}	3.86^{cde}	42.42^{cde}
6ppm MB+8 mg bentofeed	28.05^{bc}	0.024^{d}	4.11^{bcd}	43.70^{bcd}
9ppm MB	18.60^{d}	0.020^{d}	3.50^{e}	40.54^{e}
9ppm MB+4 mg bentofeed	25.69^{c}	0.023^{d}	3.91^{cde}	42.67^{cde}
9ppm MB+8 mg bentofeed	27.98^{bc}	0.024^{d}	4.17^{bcd}	44.02^{bcd}
SEM	1.53	0.002	0.17	0.88
P-value				
MB levels	<0.0001	<0.0001	<0.0001	<0.0001
bentofeed levels	<0.0001	0.53	0.024	0.024
MB × bentofeed levels	0.001	0.03	0.07	0.07

^{a,b,c,d,e} Values containing different letters in each column are significantly different (P<0.05).

b=the potential gas production (mL/200 mg DM); c=rate constant of gas production (%/h); ME=Metabolizable energy (MJ/kg DM); OMD=Organic matter digestibility (%); and MB=Methylene blue

Table 2. Effect of methylene blue with or without Persian melon peel biochar on the gas production and estimated parameters

Treatment	b	c	ME	OMD
Control	72.10^{a}	0.055^{a}	9.34^{a}	70.66^{a}
Control+4 mg PMPB	68.55^{ab}	0.055^{a}	9.01^{a}	68.96^{a}
Control+8 mg PMPB	64.09^{b}	0.053^{a}	9.04^{a}	69.14^{a}
3ppm MB	23.96^{ef}	0.027^{b}	3.96^{ef}	42.95^{ef}
3ppm MB+4 mg PMPB	31.88^{cd}	0.052^{a}	5.35^{c}	50.08^{c}
3ppm MB+8 mg PMPB	34.79^{c}	0.055^{a}	5.93^{b}	53.06^{b}
6ppm MB	17.99^{f}	0.026^{b}	3.61^{fg}	41.13^{fg}
6ppm MB+4 mg PMPB	27.89^{ed}	0.027^{b}	4.24^{de}	44.38^{de}
6ppm MB+8 mg PMPB	34.63^{c}	0.031^{b}	5.09^{c}	48.75^{c}
9ppm MB	18.55^{f}	0.021^{b}	3.47^{g}	40.42^{g}
9ppm MB+4 mg PMPB	24.67^{e}	0.020^{b}	3.68^{fg}	41.50^{fg}
9ppm MB+8 mg PMPB	34.98^{c}	0.023^{b}	4.47^{d}	45.56^{d}
SEM	1.92	0.004	0.16	0.81
P-value				
MB levels	<0.0001	<0.0001	<0.0001	<0.0001
PMPB levels	<0.0001	0.01	<0.0001	<0.0001
MB × PMPB levels	<0.0001	0.006	0.06	0.06

^{a,b,c,d,e,f,g} Values containing different letters in each column are significantly different (P<0.05).

b=the potential gas production (mL/200 mg DM); c=rate constant of gas production (%/h); ME=Metabolizable energy (MJ/kg DM); OMD=Organic matter digestibility (%); MB=Methylene blue; PMPB=Persian melon peel biochar

Table 3. Effect of methylene blue with or without bentofeed on total (mmol/L) and individual (mmol/100mol) volatile fatty acids and NH_3-N (mg/dL) concentrations

Treatment	TVFA	Acetic acid	Propionic acid	Butyric acid	NH_3-N
Control	41.87^{a}	70.75^{abc}	17.38^{abcd}	7.75	16.42^{a}
Control+4 mg bentofeed	40.77^{a}	70.87^{ab}	17.25^{bcd}	7.70	15.25^{b}
Control+8 mg bentofeed	39.00^{b}	71.62^{a}	16.47^{d}	7.78	14.87^{bc}
3ppm MB	34.17^{c}	69.17^{bcd}	18.66^{abc}	7.97	13.37^{e}
3ppm MB+4 mg bentofeed	37.70^{b}	70.50^{abcd}	17.75^{abcd}	7.62	14.75^{bc}
3ppm MB+8 mg bentofeed	39.05^{b}	71.02^{ab}	16.97^{cd}	7.89	15.42^{b}
6ppm MB	27.25^{e}	68.85^{cd}	19.22^{a}	7.78	12.20^{f}
6ppm MB+4 mg bentofeed	33.20^{cd}	69.37^{bcd}	18.85^{abc}	7.64	13.62^{de}
6ppm MB+8 mg bentofeed	34.37^{c}	70.12^{abcd}	18.11^{abcd}	7.59	14.52^{bcd}
9ppm MB	27.70^{e}	68.75^{d}	19.32^{a}	7.77	10.59^{g}
9ppm MB+4 mg bentofeed	31.62^{d}	69.12^{bcd}	19.05^{ab}	7.66	13.15^{e}
9ppm MB+8 mg bentofeed	32.50^{d}	69.87^{abcd}	18.30^{abcd}	7.64	13.95^{cde}
SEM	0.55	0.59	0.59	0.14	0.32
P-value					
MB levels	<0.0001	0.002	0.0013	0.49	<0.0001
bentofeed levels	<0.0001	0.014	0.02	0.28	<0.0001
MB × bentofeed levels	<0.0001	0.97	0.99	0.88	<0.0001

[a,b,c,d,e,f,g] Values containing different letters in each column are significantly different (P<0.05).
TVFA: Total volatile fatty acids; MB=Methylene blue

Table 4. Effect of methylene blue with or without Persian melon peel biochar total (mmol/L) and individual (mmol/100mol) volatile fatty acids and NH_3-N (mg/dL) concentrations

Treatment	TVFA	Acetic acid	Propionic acid	Butyric acid	Other	NH_3-N
Control	41.87^{a}	70.75	17.38	7.75	4.12	16.42^{a}
Control+4 mg PMPB	39.50^{b}	69.87	18.00	8.05	4.08	14.85^{bc}
Control+8 mg PMPB	37.00^{c}	70.66	17.20	8.03	4.11	13.97^{cd}
3ppm MB	34.17^{d}	69.17	18.65	8.09	4.09	13.37^{de}
3ppm MB+4 mg PMPB	36.12^{c}	70.38	17.72	7.72	4.18	14.07^{bcd}
3ppm MB+8 mg PMPB	37.25^{c}	70.12	17.95	7.77	4.16	14.97^{b}
6ppm MB	27.25^{f}	68.85	19.22	7.72	4.21	12.20^{f}
6ppm MB+4 mg PMPB	33.82^{d}	69.12	19.05	7.70	4.13	13.75^{de}
6ppm MB+8 mg PMPB	34.62^{d}	69.95	18.40	7.54	4.11	14.30^{bcd}
9ppm MB	27.70^{f}	68.75	19.32	7.83	4.1	10.59^{g}
9ppm MB+4 mg PMPB	32.32^{e}	69.87	18.29	7.67	4.17	12.92^{ef}
9ppm MB+8 mg PMPB	33.62^{de}	69.62	18.60	7.66	4.12	13.54^{de}
SEM	0.47	0.71	0.66	0.17	0.08	0.30
P-value						
MB levels	<0.0001	0.22	0.06	0.19	0.96	<0.0001
PMPB levels	<0.0001	0.37	0.64	0.67	0.96	<0.0001
MB levels × PMPB levels	<0.0001	0.78	0.88	0.57	0.92	<0.0001

[a,b,c,d,e,f,g] Values containing different letters in each column are significantly different (P<0.05).
TVFA: Total volatile fatty acids; MB=Methylene blue; PMPB=Persian melon peel biochar

4. Discussion

It has been reported that the biochar produced from palm bark, eucalyptus (Sun et al., 2013), and wood wastes (Kelm et al., 2019) can be considered a low-cost adsorbent to remove chemical dyes from aqueous solutions. As expected, higher removal of MB was observed in the increased dose (8 mg) of bentofeed and PMPB at 3 or 24 h of incubation. Similarly, an increase in MB removal (31.7% at 0.05 g/100 mL to 99.81% at 0.55 g/100 mL) was found when the lemongrass ash was increased in an aqueous solution (Singh, 2014). The higher efficiency for the removal at high doses may be attributed to the greater availability of active sites for a similar number of adsorbing molecules (Sharma et al., 2008). The adsorptive ability of chemical dye by adsorbents depends on four consecutive steps (Noroozi and Sorial, 2013).

The first step includes the diffusion or convection of dye molecules via the bulk of the solution. In the second step, the dye molecules will be filtered through a diffusional boundary layer. Furthermore, dye molecules diffuse from the surface of adsorbent materials into the interior sections, and finally, the dye molecules will reach the surface of the adsorbent via molecular interactions.

Some parameters, such as pH, initial dye concentration, adsorbent concentration, and temperature of the solution can be effective in the adsorption of dye molecules (Wee and Lim, 2016). It has been reported that 24 h is the optimum time to reaching the equilibrium point for MB dye with higher initial concentrations (400-500 mg l^{-1}) (Hameed et al., 2007). In the current experiment, two incubation times (3 and 24 h) were tested to evaluate whether or not time could affect dye removal percentage. According to Figures 1 and Figure 2, it seems that the dye removal efficiency for PMPB during 24 h of incubation is higher than 3 h. Despite the fact that the addition of bentofeed and PMPB effectively removed MB from water, the dye removal efficiency was decreased with increasing dye concentration. In this study, the efficiency of bentofeed was higher in removing MB at 9 mg/L, compared to PMPB, which can be due to the limited capacity of biochar in the removal of cationic dye because of the presence of positively charged binding sites (Fosso-Kankeu et al., 2016).

To the best of our knowledge, no research has been carried out to evaluate the effect of MB on *in vitro* ruminal fermentation so far. In the present study, the deleterious effects of MB on gas production and the estimated parameters were evident. Most chemical dyes have an aromatic complex structure that cannot be decomposed easily when releasing into the environment. In the present study, it seems that ruminal microorganisms were not able to degrade and neutralize the negative effects of MB. The bactericidal effects of chemical dyes in laboratory mediums have been previously reviewed by Hazrat (2010). Bentonite is one of the clay minerals which is used to increase the ruminant function due to its buffering capacity and ion exchange capacity (Lee et al., 2010; Kazemi et al., 2017). Lee et al. (2010) stated that the dietary addition of bentonite significantly reduced the amount of manure gas emission (SO_2, NH_3, and H_2S) of beef calves, compared to the control treatment. The addition of SB to Optigen (polymer-coated urea) had no beneficial effects on nutrient digestibility, nitrogen retention, microbial nitrogen, and rumen fermentation (Chegeni et al., 2013). In return, the use of sodium bentonite in the diet of Baluchi sheep increased the digestibility of DM and OM, compared to the control group (Kazemi et al., 2017). Decolorization of some chemical dyes has been studied using some strains of bacteria, such as *Aeromonas hydrophila*, *Pseudomonas luteola*, *Escherichia coli*, and *Pseudomonas mendocina* (Hazrat, 2010).

The rumen, as an essential fermentation chamber, produces end products (e.g., VFAs, NH_3-N, and microbial protein) to resolve the energy and protein requirements of the host animal (Rindsig et al., 1969). Therefore, any changes in living conditions of microbial communities can alter the fermentation pattern or gas produced in the medium. Bentonite can modify the rumen fermentation pattern by reducing the propionate relative to acetate (Rindsig et al., 1969). In this study, the propionate and acetate levels were decreased and increased with increasing levels of bentofeed in the medium, respectively, which was consistent with the results of a previously conducted study.

Similar to our results, Cabeza et al. (2018) found a reduction in gas production by adding biochar prepared from different biomass sources at 1.16% of substrate DM. This depressing effect may be attributed to the sorption capacity of biochar because of its high porosity structure (Hansen et al., 2012). However, most of the previous studies have shown that the addition of biochar did not affect total gas production (Leng et al., 2012; Calvelo Pereira et al., 2014). In addition to other factors, such as dosage and post-treatment of biochar, different biomass sources and temperature can affect *in vitro* responses to biochar (Calvelo Pereira et al., 2014). Kumar et al. (1987) reported that increasing pH, and therefore, providing favourable habitat for cellulolytic bacteria following the inclusion of charcoal to anaerobic batch fermenters stimulated their growth and activity. Since ammonia, as the only N source, is essential for the growth and activity of cellulolytic bacteria (Atasoglu et al., 2001), partial reduction in NH_3-N concentration in our study might be due to an increase in rate and/or extent of microbial growth.

In vitro response to the addition of biochar is still controversial. The inclusion of different sources of biochar with different doses had no effect on total VFA production (Cabeza et al., 2018). However, Calvelo Pereira et al. (2014) reported that total VFA production was increased as the concentration of biochar in the silage increased from 8.11% to 18.6% of DM; however, total VFA and molar proportion of individual VFA did not change when biochar was mixed with hay at 16% of diet DM.

In conclusion, PMPB and bentofeed can be used to reduce the high amounts of MB from contaminated water, and also can partially reduce the deleterious effects of MB on the medium. The biochar produced from melon peel appears to be a promising adsorbent to remove MB, not only for the high efficiency in removing the dye but also due to the advantages from the environmental point of view.

Authors' Contribution

Study concept and design: M. K.
Acquisition of data: M. K.
Analysis and interpretation of data: M. K. and A. M.
Drafting of the manuscript: M. K. and A. M.
Critical revision of the manuscript for important intellectual content: M. K. and A. M.
Statistical analysis: M. K.
Administrative, technical, and material support: M. K.

Ethics

The authors declare that all ethical standards have been respected in the preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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